DNA Structure and Dynamics I

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Mechanical Unfolding of Human Telomere G-Quadruplex DNA Probed by Integrated Fluorescence and Magnetic Tweezers Spectroscopy

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Telomeres are specialized chromatin structures that protect chromosome ends from nucleolytic processing by DNA repair machinery. The foundation of human telomere structure is a long array of tandem DNA sequences (TTAGGG), which can fold into a class of secondary structures known as G-quadruplexes (GQ). Previous studies revealed that GQs are highly polymorphic and a variety of topologically distinct forms may coexist under a single folding condition[1-3]. Single molecule Förster resonance energy transfer (smFRET) experiments demonstrated the dynamic nature of GQ structure, and suggested that interconversion between topologically distinct GQ folds proceeds through an obligatory transient intermediate[4]. To further characterize this GQ folding intermediate we developed employed an integrated fluorescence and magnetic tweezers spectroscopy technique, which permits the application of a wide range of stretching forces (0.1-50 picoNewtons) to individual GQ folds, together with simultaneous detection of GQ folding and unfolding through smFRET. Here, we present our investigation of the Na^+-induced antiparallel GQ conformation. Analysis of the force-dependent rate constants for the GQ folding and unfolding reactions provided an estimate of the position of transition state for GQ unfolding along the DNA stretching coordinate. The results suggest that telomere GQ is sensitive to mechanical force; only small perturbations can disrupt the entire structure. Furthermore, by comparing the GQ unfolded state with a single-stranded polyT DNA we show the unfolded GQ exhibits a significantly compacted non-native conformation reminiscent of the protein molten globule.

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G-Quadruplex DNA Folding and Dynamics within Duplex DNA

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The helix is the classical view of genomic DNA structure. Recent findings suggest that alternate folding motifs can play an important role in gene expression. One example is the G-Quadruplex, a single stranded DNA sequence composed of four repeat sequences of two or more G's each separated by varying spacer sequences. This structure has been suggested to affect the expression of several important genes in cancer development (c-MYC, c-KIT, VEGF)[1].

Recently, immune assay based experiments have shown that G-Quadruplexes are stably found throughout the genome [2]. The dynamic properties underlying the interaction between the G-Quadruplex and its single stranded DNA complement have yet to be characterized. Preliminary studies suggest that these structures are capable of forming in response to specific cellular actions and are persistent in a biologically relevant time scale.

To determine sequence dependent properties of G-Quadruplexes, we utilized a combination of Single Molecule FRET microscopy and small molecule binding fluorescence assays. We investigated biophysical properties such as stability, folding patterns, and structure persistence. These properties will help elucidate the biological roles of specific genomic quadruplexes. This knowledge will aid in interpreting the roles of the many G-Quadruplex associated proteins and may assist in the development of the next generation of chemotherapeutic agents.

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[2]. Lam, EYN et al. (2013) Nature Communications USA 4:1796.

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Validation and Physical Characterization of Ribosomal G-Quadruplexes with MD Simulations

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DNA and RNA adopt structures known as G-quadruplexes, and they are typically found in the telomere regions of chromosomes and in promoter regions. G-quadruplexes that form in gene promoter regions are targeted at the nucleolus, the site of ribosome biogenesis, and its formation and stabilization in the nucleolus can act as silencers in gene expression, control cell growth, and thus provide a viable treatment for cancer. Although it is well-known that ribosomal sequences are guanine-rich and could potentially form G-quadruplexes, these putative sequences are seldom validated. In our study, we modeled two putative parallel DNA quadruplex sequences that are based on structures of known stable parallel and antiparallel DNA quadruplexes. We performed 20 ns atomistic molecular dynamic simulations with the CHARMM36 force field and the NAMD simulation suite. We then calculated the base-base distances and pseudo-torsion angles to project these quantities to free energy profiles for the purpose of determining their relative stabilities. Based on these simulations, we created a conformational and structural map based on the free energy stabilities of two putative quadruplex sequences, and they can serve as the basis of developing selective anti-cancer agents.

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G-Quadruplex Folding Depends on its Loop Size and Sequence: Extreme Fast Folding Kinetics Observed in Human Telomere and its Isomer

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Highly stable GQ structures have been observed at telomeres and GC rich promoter regions of several oncogenes in human cancer cells. Potential GQ forming sequences entail triplet Gs (GGG) separated by variable sequence and length of none-G bases, termed loop which often determines GQ folding conformation. Folding conformations of GQ structures play an important role in targeting GQ binding anticancer drugs with high specificity and selectivity. It is very challenging to quantify each folded population accurately using bulk methods. Ensemble measurements only provide information on existence of both folded populations. Here we employed single molecule FRET to observe two GQ folding conformations (parallel, antiparallel) and unfolding in real time. In order to understand nature of GQ folding kinetics, we varied the loop length and sequence systematically in between triplet Gs. Our result reveals that a single base in one loop between any triplet Gs instantly drives GQ folding into parallel conformation. Furthermore, keeping two or more bases leads to appearance of antiparallel conformation first then it interconverts between other conformations at equilibrium. We quantified the fraction of each conformation from FRET intensity histograms. Furthermore we explored the loop sequence variance in the context of human telomere (TTA) and its isomers (TTT, TAA, AAA). Interestingly, kinetic analysis indicates that the human telomeric sequence exhibits extremely fast folding rates compared to its counterparts. Higher preference for either of the folded state observed in human telomere and its isomers could have implication in protecting genome by tightly capping chromosome end. Observed extreme rates of GQ folded state could potentially useful in targeting GQ binding anticancer drugs at the telomere regions.

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DNA i-MOTIF Probed by Photoacoustic Calorimetry

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The intercalated motif of DNA occurs in the C-rich strand of regulatory regions of the genome and in the human telomere, where it competes with the G-quadruplex and the DNA duplex. Its formation is strongly favored at acidic pH (<6). The folding rates for various forms of i-motif have been determined previously, but the initial steps of folding are not well characterized. Photoacoustic calorimetry (PAC) has been used in conjunction with a 2-nitrobenzaldehyde pH-jump technique to characterize thermodynamic and kinetic parameters associated with the protonation and initiation of the intramolecular i-motif folding' on fast timescales (~50ns to 10μs). Two kinetic steps were resolved, corresponding to the intramolecular tautomerization of 2-nitrobenzaldehyde and cytosines protonation within 50 ns. This is followed by nucleation of i-motif folding with ~300 ns lifetime. We determined ΔH and ΔV for the fast step to be −74.0 kcal mol^{-1} and −6.1 mL mol^{-1} in the range of 7 - 20°C; from 20-30°C the values are 99.7 kcal mol^{-1} and 25 mL mol^{-1}. Photothermal beam deflection data indicate the absence of kinetic steps between ~10 μs and 10 ms. In addition, the pK_a and stability of the i-motif structure with respect to temperature have been probed by circular dichroism. At pH<7, the fraction of i-motif folded shows little temperature dependence in the range of 7-30°C, whereas at pH 7 the fraction folded decreases from 0.19 to 0.08. The temperature dependence of i-motif folding close to biological pH and temperature has potential implications for the role of i-motif *in vivo*.

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Thermodynamics of the G-Quadruplex Formation of Modified Human Telomeric Sequences

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Oligodeoxyribonucleotides (ODNs) that are rich in guanine can form four stranded structures known as G-quadruplexes. These structures are stabilized